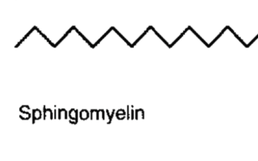
Characterization of Vacuolating cytotoxin A binding to sphingomyelin in *Helicobacter pylori* pathogenesis

Seokjoo Choi, Hyunjung Anna Kim, Seung Jin Oh, and Steven R. Blanke
Department of Molecular and Cellular Biology, School of Molecular and Cellular Biology, University of Illinois at Urbana-Champaign

Abstract

The main objective of my research project is to characterize vacuolating cytotoxin A (VacA) from Helicobacter pylori binding to an important host cell membrane lipid, sphingomyelin (SM). Previously, our laboratory showed that plasma membrane SM is important for the toxin biological activity, cell surface binding, and toxin-receptor direct interactions suggesting that SM is a receptor for VacA. Moreover, recent findings from our laboratory showed that R552, W603, and R647 of VacA are important that when changed to alanine, resulting in decreased SM-dependent VacA activity in gastric epithelial cells. However, the molecular basis of SM-VacA interactions remains unknown. My research focuses molecular on the detailed molecular mechanism by which these three residues of VacA interact with SM in SM-dependent toxin cellular activities. I will evaluate the hypothesis that R552, W603, and R647 on VacA facilitate its SM binding by interacting with the phosphorylcholine head group of SM. To test this hypothesis, I will conduct site-directed mutagenesis analysis to evaluate the specific properties of the three residues (R552/W603/R647) that are important for SM-dependent toxin cellular activities. I will evaluate the prediction that VacA interacts with SM through pi-cation interactions between the aromatic ring of tryptophan and choline moiety of head group of SM and ionic interactions between positively charged arginine residues and negatively charged phosphate moiety of SM. Testing this prediction, I am evaluating the toxin cellular activity of charge conservative and non-conservative single substitution mutations in the three residues. The results of this study will provide the framework for the molecular interactions behind VacA-SM interactions.

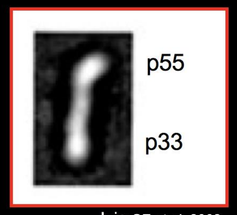
Helicobacter pylori



(Microwiki)

- Colonize in stomach
- ~50% of world population is infected
- Persistent infection increases risk of gastric diseases including gastric peptic ulcer and gastric adenocarcinoma
- Gastric adenocarcinoma is third leading cause of cancer related deaths (~723,000 deaths in 2012)

Vacuolating cytotoxin (VacA)



Ivie *SE et al.,2008*

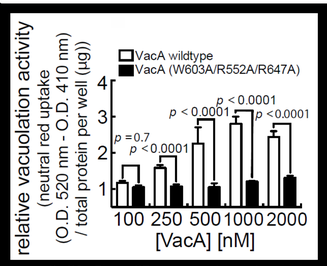
Cover et al. 138 (4): 759

- Intracellular acting exotoxin
- Membrane channel activity
- Important for *H. pylori* colonization and pathogenesis

Sphingomyelin (SM) functions as a receptor for VacA

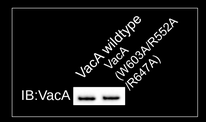
Kolesnick, R., & Golde, D. W., 1994, *Cell,* 77(3), 325-328

VacA (R552A/W603A/R647A) showed significant attenutation in toxin activity

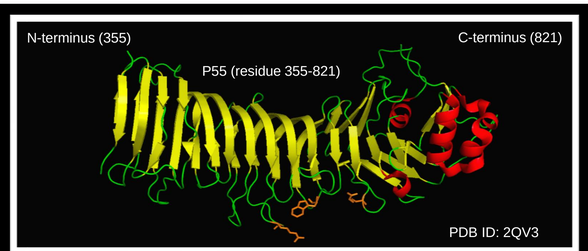


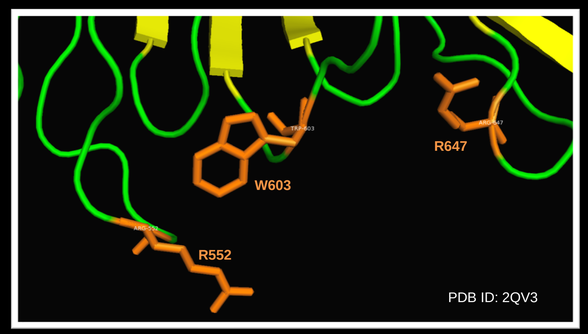
Oh SJ., Blanke SR, manuscript ready to publish

Cell types: AZ-521 cells
Seeding cell number : 3*10^6 cells/mL
[VacA] (nM): 100, 250, 500, 1000, 2000
Intoxication time: 16hr, pulse exposure
Assays: Neutral Red Uptake and BCA assay

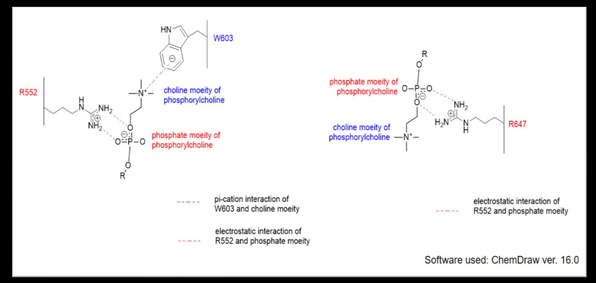


Molecular basis of the interactions between R552, W603, and R647 of VacA and SM is unknown



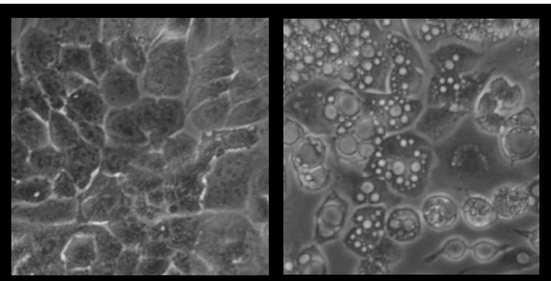


Hypothesis: R552, W603, and R647 of VacA facilitate its SM binding by interacting with phosphorylcholine headgroup of SM



Kim, HA., Oh SJ., Blanke SR, 2017, unpublished

VacA induces vacuolation in intoxicated cells



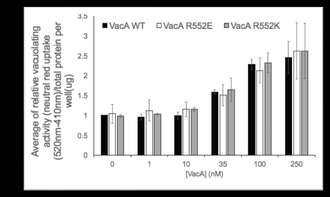
No VacA VacA (250nM)

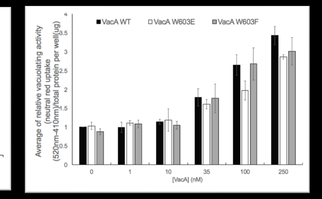
AZ-521 cells: Human derived esophageal epithelial cells

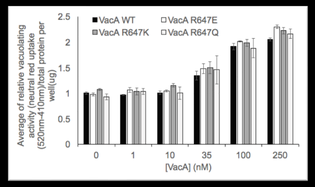
Conservative and non-conservative mutations of R552, W603, and R647 of VacA

Residue	Mutation	Mutation type	Prediction
R552	R552E	Non-conservative mutation	Negative charge of **Glutamate** possibly disrupts the electrostatic interaction with phosphate moiety of SM
	R552K	Conservative mutation	Positive charge of **Lysine** possibly interacts with negatively charged phosphate moiety of SM
	R552Q	Conservative mutation	Amide group of **Glutamine** provides hydrogen bonding opportunity with phosphate moiety of SM
W603	W603E	Non-conservative mutation	Negative charge of **Glutamate** may or may not interact with positively charged choline moiety of SM
	W603F	Conservative mutation	Phenyl group of **Phenylalanine** may form pi-cation interaction with choline moiety of SM
	W603K	Non-conservative mutation	Positive charge of **Lysine** possibly interacts with negatively charged phosphate moiety of SM
	W603S	Non-conservative mutation	Hydroxyl group of **Serine** provides hydrogen bonding opportunity with phosphate moiety of SM
	W603Y	Conservative mutation	Phenyl group of **Tyrosine** may form pi-cation interaction with choline moiety of SM, Hydroxyl group of **Tyrosine** provides hydrogen bonding opportunity with phosphate moiety of SM
R647	R647E	Non-conservative mutation	Negative charge of **Glutamate** may disrupt the electrostatic interaction with phosphate moiety
	R647K	Conservative mutation	Positive charge of **Lysine** possibly interacts with negatively charged phosphate moiety of SM
	R647Q	Non-conservative mutation	Amide group of **Glutamine** provides hydrogen bonding opportunity with phosphate moiety of SM

Mutant VacA showed similar biological activity to WT VacA

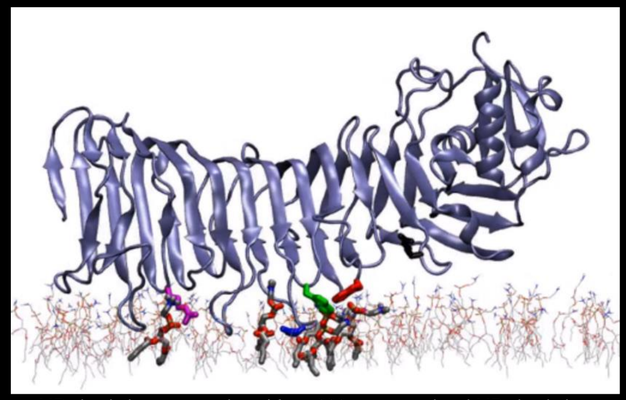






Cell type: AZ-521 (seeded 2.0X10^6 cells/ml)
[VacA]: 0, 1, 10, 35, 100, 250nM, pulse
VacA binding: 30min, Intoxication time: 16hr
Method: Neutral red uptake and BCA assays
Three independent studies were combined and error bar represent standard deviations

Future direction



gaMD simulation was continued from 390ns conventional MD simulation (Paween M., et. al., 2017, unpublished)

- All the single VacA mutants will be tested for their toxin activity.
- Further site-directed mutagenesis will be conducted on loops and test for their toxin activity.
- Understanding of molecular basis of VacA-SM interactions in SM-dependent toxin binding and cellular activity

Acknowledgments

- National Institute of Health (R01 AI045928)
- 2018 School of MCB Summer Undergraduate Research Fellow
- Blanke Lab members